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Original Research

Optimization and Validation of Kinetic-Spectrophotometric Technique for the Determination of Pesticide Dicamba in Infant Baby Foods Using Solid Phase Extraction Method

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Abstract

The objective of this paper was to develop and apply the kinetic-spectrophotometric method for the determination of the pesticide dicamba in infant baby foods available in Serbia. The method is based on the inhibition effect of dicamba on the oxidation of sulfanilic acid (SA) by hydrogen peroxide in universal buffer (pH = 9.66) in the presence of Co^{2+} ion. The reaction was monitored spectrophotometrically at 368 nm. The HPLC method was used as a comparative method to verify the results of the kinetic method. Under the experimental conditions proposed, dicamba showed a linear dynamic range of 0.31 to 3.10 μg mL^{-1}, and from 3.10 to 31.00 μg mL^{-1} with standard deviation from 1.77 to 4.55 %. Limit of detection and a limit of quantification are 0.101 μg mL^{-1} and 0.306 μg mL^{-1}, respectively. The kinetic method was efficiently applied for dicamba determination in baby food samples after solid phase extraction. This method is highly sensitive, simple, easy to operate, and requires cheap reagents. It can be successfully used for routine analysis of dicamba in baby food.

Keywords: dicamba, kinetic method, SPE, HPLC, baby food samples

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Introduction

The worldwide, steady expansion of population demands increases in food production, attention to public health, and protection of the environment. Pest control is an integral part of the development of every country. Pests harm crops and transmit diseases. Since chemical control of pests is so successful, there has been an explosive expansion in the development of synthetic organic pesticides. Except for the organochlorine compounds, most of these chemicals persist for only a few weeks or months in the environment. However, as a result of the continued use of pesticides, appreciable quantities of pesticide residues and their degradation products accumulate in the biota [1]. Pesticides are widely utilized at various stages of cultivation and during postharvest storage to protect plants against a range of pests and/or to provide quality preservation. Pesticides are commonly found in baby food consumed by infants in the first year of life. The levels detected are typically well below federal standards, but these standards do not specifically incorporate any special protections for infants or young children.

Dicamba (Fig. 1) is a selective systemic herbicide, absorbed by the leaves and the roots, with ready translocation through the plants *via* both the sympatic and apoplastic systems. It is used in agriculture against annual and perennial broad-leaved weeds and brush species. Dicamba belongs to the group of Phenoxyacid herbicides (PAs). Due to their strong polarity PAs are the most likely to leach to ground water and soil, ultimately polluting soils and groundwater. Their mobility through agricultural ecosystems leads to the contamination of the environmental surface and waters. On the other hand, they can enter human body through contaminated drinking water. Moreover, these herbicides will remain in the crops which are used for food products or animal feeds when they are applied during the period of spraying prohibition and eventually enter either human or animal body through the food chain. They can cause soft tissue carcinoma in humans and embryotoxicity in animals [2].

Pesticides are pollutants that can be found on the surface of fruits and vegetables. Increased concentrations of these pollutants significantly affect human health. For this reason, there is a constant need for their monitoring in food for infants use. Commercially available infant foods have become an important part of the diet of many infants and toddlers because of their mineral and vitamin content that fulfills dietary requirements. World Health Organization [3] and American Society of Pediatrics [4] reports recommend exclusive breastfeeding for the first 6 months of life; however, sometimes breast feeding is not sufficient [5], or after six months feed, the complementary feeding becomes necessary. It is obvious that infant baby food produced by different producers have not been fully and comprehensively investigated to determine the trace and residue of pesticides, especially in commercial infant cereal formula. Therefore, the need for the routine monitoring of infant food products can not be neglected. We have carried out the work in order to have an up to date knowledge on the infant baby food. Reliable confirmatory methods are required to monitor pesticide residues in baby foods and to ensure the safety of baby food supply.

The most suitable approaches in the determination of the pesticide residue contents in food samples are chromatographic methods with various sample preparation methods. A considerable number of extraction procedures were developed and applied for the preconcentration and prepurification of phenoxy acid herbicides [6]. So far, a number of classic extraction methods such as liquid-liquid extraction (LLE) [7], solid-phase extraction (SPE) [8, 9], solid-liquid extraction (SLE) [10, 11] transfer microextraction (TME) [12] and dispersive liquid-liquid microextraction (DLLME) [13-17] for sample pretreatment of PAs have been developed and reported. Analytical techniques used in the determination of pesticides are mainly High-performance liquid chromatography (HPLC) [18] and Ultra-high performance liquid chromatography (UHPLC) method [19-21] or Gas Chromatography (GC) coupled to selective detection systems, such as an mass spectrometric detection (MS) [22-27]. Several variations of the QuEChERS method have been investigated and evaluated for the extraction and cleanup of various PAs from complex matrices such as foods, feeds, and waste. Modified QuEChERS extraction procedure coupled with quantitation by liquid chromatography tandem mass spectrometry (LC-MS/MS) [28-30] or GC-MS/MS methods [31]. The capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC) metods are commonly used in the analysis of phenoxy acid herbicides [32-34]. Ultra high-performance liquid chromatography and electrospray ionization quadrupole Orbitrap high-resolution mass spectrometry (UHPLC-ESI Q-Orbitrap) was developed by Jia and authors [35] for the separation and detection pesticides in baby food analytes. Petrarca et al. developed d-SPE and DLLME methods followed by GC method for pesticide analysis in baby foods [36]. In the literature, many chromatographic methods have been reported for the quantification of dicamba in environment [37] and there is only one report of simultaneous determination of dicamba in the range of 0.8-8.8 μg mL^{-1} and with a limit of detection 0.3 μg mL^{-1} by UV-Vis spectrophotometry reported by Hernández and coworkers [38].

The main aim of the present work was to develop a simple, selective and sensitive method for the quantitative determination of dicamba by a kinetic spectrophotometric method, and also, to apply a new method for dicamba determination in baby food samples after preparation of food samples by SPE. The method was based on the inhibition effects of dicamba on the oxidation reaction of sulfanilic acid (SA) with H_2O_2 in alkaline media in the presence of Co^{2+} ions, monitored at λ of 368 nm. The differential variant of tangent method

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was used for data processing. The HPLC method was used like an comparative method to verify the results of kinetic method.

Material and Methods

Dicamba standard was purchased from Dr Ehrenstorfer (Germany) with a certified purity of 99%. Standard stock solutions containing 400 μg mL^{-1} of dicamba were prepared by dissolving the required amounts of the standards in methanol. They were stored in a refrigerator at 4ºC. Working solutions were prepared by diluting the stock solutions with methanol-water (50/50, v/v). A $4 \cdot 10^{-2}$ mol L^{-1} solution of the substrate sulfanilic acid (p. a. Merck, Germany) was prepared by dissolving 0.3463 g of a SA in 50 mL of water. The initial 2 mol L^{-1} solution of hydrogen peroxide was prepared from 30% H_2O_2 (p. a. Merck, Germany), and its exact concentration was standardized permanganometrically. Because of their limited stability, it was prepared just before use. 0.01 mol L^{-1} stock solution of Co^{2+} ions was prepared by dissolving $CoCl_2 \cdot 2H_2O$ (p. a. Merck, Germany) in 100 mL of water. Its exact concentration was controlled complexometrically. The universal buffers [39] in pH interval 8.00-10.00 were obtained by mixing solutions of CH_3COOH (0.4 mol L^{-1}), H_3PO_4 (0.4 mol L^{-1}), H_3BO_3 (0.4 mol L^{-1}) with NaOH (0.1 mol L^{-1}). Chromatographic grade methanol (MeOH) and dichlormethane (DCM) were purchased from Baker (UK). Potassium dihydrogen phosphate (KH_2PO_4) was purchased from Merck (Darmstadt, Germany). Analytical grade chemicals and deionised water (MicroMed high purity water system TKA Wasseraufbereitungssysteme GmbH) were used for the preparation of all solutions.

A Perkin-Elmer Lambda UV/Vis spectrophotometer (USA) with 10-cm quartz cell pairs was used for recording the absorbance at 368 nm. A water bath thermostat (n-BIOTEK, INC, model NB-301, Korea) was employed to control the reaction temperature. A stopwatch (TQC SHEEN, Germany) was used to record the reaction time.

Chromatographic analyses were performed with an Agilent Technologies (USA), Series 1200 liquid chromatograph, equipped with an Agilent photodiode array detector (DAD), Model 1200 with RFID tracking technology for flow cells and an UV lamp, an automatic injector and Chem Station software. The analytical column was an Agilent – Eclipse XDBC-18 C_{18} column (150 × 4.6 mm).

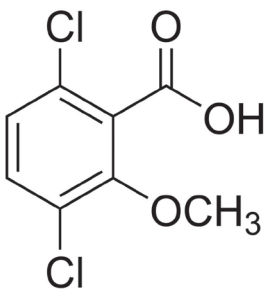

Fig. 1. Chemical structure of dicamba.

A solid phase extraction system (J. T. Baker Model SPE-12, UK) with a vacuum pump was used for solid phase extraction of samples. SPE with Chromabond® HR-P cartridges (sorbent mass 200 mg, Macherey Nagel, Germany) were used for extraction of dicamba.

A rotary vacuum evaporator (model BÜCHI R-200/205, Switzerland) including bath B-490 with a vacuum pump was used to evaporate the extracts.

An analytical balance (Mettler Toledo, USA) was used to measure the mass.

Hanna pH-meter instrument was used for the pH measurements.

The solutions were thermostated at 25±0.1ºC before the beginning of the reaction in Julabo MP-SA, Germany model thermostatic bath.

A standard bench-top homogenizer (Model PT 2100 Polytron, Fisher Scientific, UK) was used for blending the samples.

Kinetic Procedure

The reaction was performed in a special glass four-compartment reaction vessel-mixer with lapped flap. An aliquot solution of SA was transferred into first compartment of vessel; the second was filled with buffer solution; third with Co^{2+} ions and dicamba and the fourth with H_2O_2 solution, and completed to volume 10 mL with deionised water. The mixer-vessel was kept 10 min at temperature of 25±0.1ºC. The solutions were mixed and homogenized by shaking and then transferred into 10 cm constant temperature cell of spectrophotometer. The absorption at 368 nm was read over a period of 6 min. The rate of the reactions at different concentrations of reactants was obtained by measuring the slope of the linear part of kinetic curve to the absorbance – time plot. The calibration graphs were obtained by tangent method under the optimum conditions.

Samples Collection and Preparation

Twenty-five commercially available infant baby foods were used for the optimization and validation of the analytical method. All samples were commercially available from markets in Niš, Republic of Serbia. For the real sample analysis, infant baby foods of different brands produced by different companies were purchased in 2019 in local supermarkets. The baby food was divided into three groups depending on the age of child who consumed the food: by the fourth month of life, by the sixth month of life and by the eighth month of life. Baby foods were made of rice, corn and different type of grains; some of them were prepared by the addition of fruit, vegetable or caramel.

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Baby food samples for recovery determination were prepared by dissolving 2 g in 20 mL of water, then the appropriate amount of standard stock solution (100 mg L^{-1}) was added and the obtained mixture was left for one day. The sample was homogenized with 30 mL of methanol in separating funnel and shaken with 60 mL DCM divided in three portions of 20 mL. Two distinct layers were formed; the lower organic layer was transferred into a separating funnel and decanted through anhydrous Na_2SO_4. The organic layer was further extracted with DCM, decanted through anhydrous Na_2SO_4 and combined with the organic fraction from the first extraction. After liqud-liquid extraction filtrate was treated by solid phase extraction on Chromabond® HR-P cartridges. Each sample solution was poured into a cartridge which had been conditioned with 20 mL CH_3COOH (1 mol L^{-1}), then 6 mL of deionised water, 3 mL of MeOH, and column was dried for 30 min under the gentle nitrogen steam. The sample was eluted with 3 mL MeOH and then eluted with 2 mL K_2HPO_4 (0.1 mol L^{-1}). The extract was collected and evaporated at 60ºC in a rotary vacuum evaporator till dryness. The residue was dissolved with MeOH and transferred into volumetric flask (25 mL), and divided into two parts. One part of the solution was filtered through a 0.45-µm microporous nylon membrane (Sigma – Aldrich, USA), then it was transferred into vials for HPLC analysis. An equivalent of 20 µL was injected into the HPLC system. The mobile phase was MeOH-water (50:50, v/v) delivered at a flow-rate of 1 mL min^{-1}. The analytical column was an Agilent – Eclipse XDBC-18 C18 column (150 × 4.6 mm) with diode array detection at λ of 210 nm operating at 25ºC. For each sample this procedure was carried out in triplicates.

The second part of the solution was used for kinetic determination; 10 mL of this solution was transferred into rotary evaporator and evaporated to near dryness (controlled at 60ºC). The residue was dissolved in methanol transferred in (10 mL) volumetric flask and made up with water to a final volume of 10 mL and used for kinetic determination. For each sample this procedure was carried out in triplicates.

Results and Discussion

Kinetic Studies

A yellow colored product with maximum absorption at 368 nm was formed when sulfanilic acid was allowed to react with H_2O_2 in alkaline media in presence of Co^{2+} ions as a catalyst. The initial experiments showed that when the dicamba was added to the reaction mixture it shows an inhibition effect. To determine the lowest possible determinable concentration of dicamba, working conditions were needed to be optimized. Therefore, the dependence of the rate of reactions on the concentration of each of the reactants was determined.

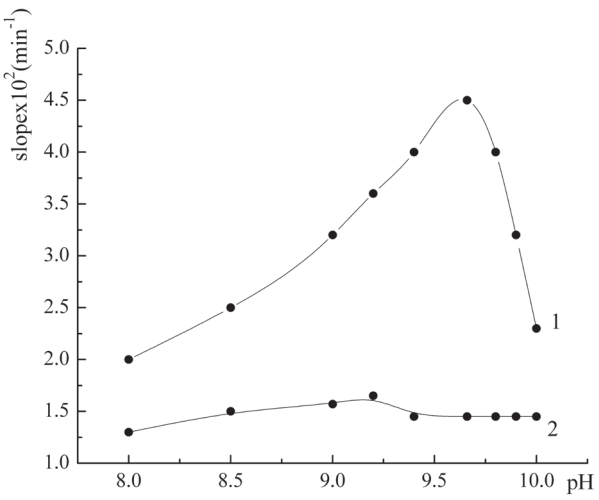

Fig. 2. Dependence of the reaction rate on the pH for the catalytic (1) and inhibited (2) reaction. Initial concentrations: c(SA) = $4 \cdot 10^{-3}$ mol L^{-1}; c(H_2O_2) = 0.25 mol L^{-1}; c(Co^{2+}) = $7 \cdot 10^{-5}$ mol L^{-1}; c(Dicamba) = 31 µg mL^{-1}; t = 25.0±0.1ºC

A tangent method was used to process the kinetic data. The reaction rate was obtained by measuring the slope of the linear part of the kinetic curve of the absorbance-time plot (slope = dA/dt).

In Fig. 2. the influence of pH on the initial rate in the presence and absence of dicamba is shown. The influence of pH on the reaction rates was studied in the interval pH from 8.0 to 10.0. It can be seen that catalytic reaction rate increases with increasing of pH and rises to pH 9.66, then decreases. Reaction rate of inhibited reaction reaches a maximum at pH value of 9.00, than it decreases. For further work a pH value of 9.66 was used. Catalytic reaction is – 0.97 order in the interval pH 8.0-9.66, and the inhibited reaction is minus

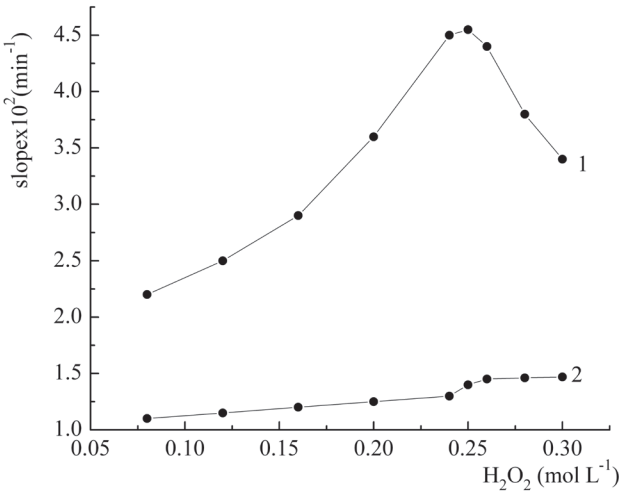

Fig. 3. Dependence of the reaction rate on the H_2O_2 concentration for the catalytic (1) and inhibited (2) reaction. Initial concentrations: pH = 9.66 ; c(SA) = $4 \cdot 10^{-3}$ mol L^{-1}; c(Co^{2+}) = $7 \cdot 10^{-5}$ mol L^{-1}; c(Dicamba) = 31 µg mL^{-1}; t = 25.0±0.1ºC.

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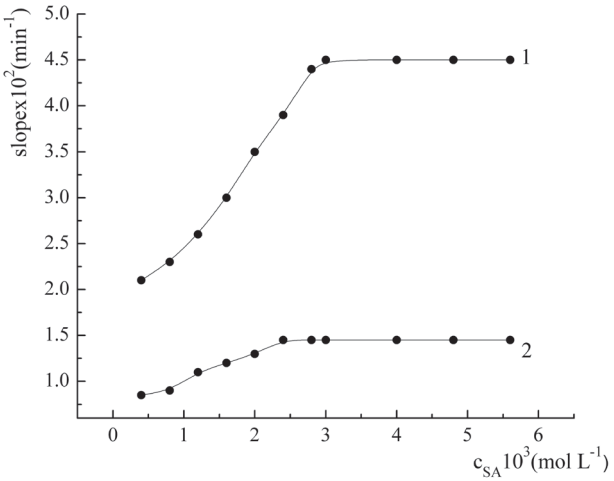

Fig. 4. Dependence of the reaction rate on the SA concentration for the catalytic (1) and inhibited (2) reaction. Initial concentrations: pH = 9.66; $c(H_2O_2)$ = 0.25 mol L^{-1}; $c(Co^{2+})$ = $7 \cdot 10^{-5}$ mol L^{-1}; c(Dicamba) = 31 μg mL^{-1}; t = 25.0±0.1ºC.

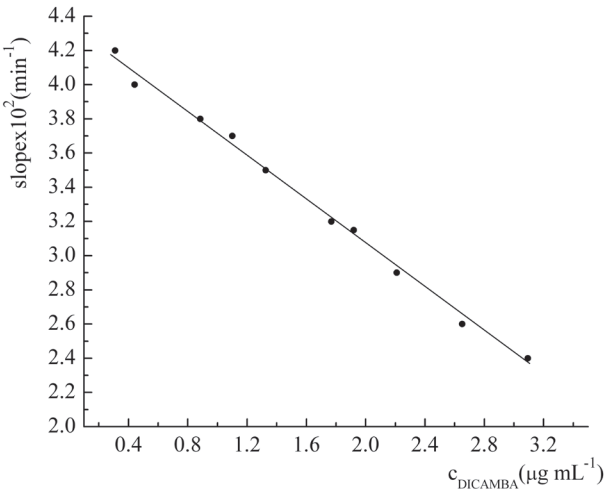

Fig. 6. Dependence of the reaction rate on the dicamba concentration in the interval 0.31-3.10 μg mL^{-1}. Initial concentrations: pH = 9.66; $c(H_2O_2)$ = 0.25 mol L^{-1}; c(SA) = $4 \cdot 10^{-3}$ mol L^{-1}; $c(Co^{2+})$ = $7 \cdot 10^{-5}$ mol L^{-1}; t = 25.0±0.1ºC.

first order (–1) in the interval pH 8.0-9.0, and zero order in the interval pH from 9.0 to 10.

The dependence of the initial reaction rate on the H_2O_2 concentration is shown in Fig. 3. It can be seen that catalytic reaction increases with the increasing of H_2O_2 concentration and reaches the maximum at concentration of H_2O_2 0.25 mol L^{-1}. The inhibited reaction increases over the investigated interval of concentration. The 0.25 mol L^{-1} concentration of H_2O_2 was chosen as optimal. Catalytic reaction is first order in the interval H_2O_2 0.04 – 0.25 mol L^{-1}, and inhibited reaction is first order through the whole investigated interval.

Influence of the SA concentration in the interval $0.4 \cdot 10^{-3}$-$6.0 \cdot 10^{-3}$ mol L^{-1} on reaction rates is shown in Fig. 4. The reaction rates increase to SA concentration of $2 \cdot 10^{-3}$ mol L^{-1} and then reach a saturation plateau. The SA concentration of $4 \cdot 10^{-3}$ mol L^{-1} was chosen as optimal for further work. Both reactions are zero order.

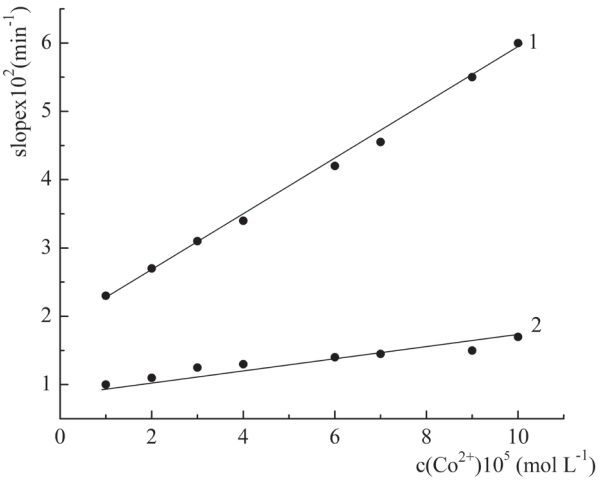

Fig. 5. Dependence of the reaction rate on the Co^{2+} ion concentration for the catalytic (1) and inhibited (2) reaction. Initial concentrations: pH = 9.66; $c(H_2O_2)$ = 0.25 mol L^{-1}; c(SA) = $4 \cdot 10^{-3}$ mol L^{-1}; c(Dicamba) = 31 μg mL^{-1}; t = 25.0±0.1ºC.

The correlation between slope and the Co^{2+} ions concentration is given in Fig. 5. The initial rates of the catalyzed and inhibited reactions rates were examined in the range $1 \cdot 10^{-5}$-$9 \cdot 10^{-5}$ mol L^{-1}. The concentration of $7 \cdot 10^{-5}$ mol L^{-1} was used as optimal through the experiment. Both reactions are the first order through the whole investigated interval of Co^{2+} ions. The value of $7 \cdot 10^{-5}$ mol L^{-1} was used as optimal concentration of the metal.

Under the optimum reaction conditions, a linear calibration graph of dicamba was obtained in the interval 0.31-3.10 μg mL^{-1} and 3.10-31.0 μg mL^{-1}. Fig. 6. shows the calibration curve at the temperature of 25.0ºC, which can be used for the determination of the dicamba concentration in the interval 0.31-3.10 μg mL^{-1}.

The equations of the calibration graphs were:

$$\text{Slope} \cdot 10^2 = -0.0337 \cdot c_{\text{Dicamba}} + 2.50 \qquad r = -0.9977 \quad (1)$$

$$\text{Slope} \cdot 10^2 = -0.646 \cdot c_{\text{Dicamba}} + 4.36 \qquad r = -0.9975 \quad (2)$$

...where c_{Dicamba} is concentration of insecticide expressed in μg mL^{-1}. The detection limit of dicamba was 0.101 μg mL^{-1}.

The kinetic equations for the catalyzed and inhibited reaction were deduced based on obtained graphic correlations:

$$Rate_I = k_1 \cdot c(H^+)^{-0.92} \cdot c(H_2O_2) \cdot c(Co^{2+}) \qquad (3)$$

Table 1. Accuracy and precision of dicamba determination.

Added (μg mL^{-1})	Determined[a] $\bar{x}$ ±SD (μg mL^{-1})	n	RSD (%)	G (%)	$\frac{\bar{x}-\mu}{\mu}\cdot 100$ (%)[b]	Recovery (%)
0.31	0.33±0.02	5	6.10	9.42	6.45	106.45
1.32	1.29±0.03		2.33	1.10	-2.27	98.38
3.10	3.05±0.07		2.30	0.93	-1.63	97.72

[a] Mean and standard deviation of five determinations at the 95 % confidence level; n- number of replicates; RSD - relative standard deviation; G- relative error; [b] accuracy of the method

$$Rate_{II} = k_2 \cdot c(H^+)^{-1} \cdot c(H_2O_2) \cdot c(Co^{2+})\, c(Dicamba)^{-1} \quad (4)$$

...where k_1 and k_2 are constants proportional to the rate constant of the catalyzed and inhibited reaction, respectively.

Validation Parameters

The proposed method has been validated for linearity, precision, accuracy, recovery and selectivity.

For evaluation of linearity, determination of dicamba was done at ten concentration levels for each calibration curve (0.31-3.10 μg mL^{-1} and 3.10-31.00 μg mL^{-1}) and it was assessed by the correlation coefficients. Each measurement was repeated five times.

In order to evaluate the accuracy and precision of the method, three concentrations of dicamba from the calibration curve were selected. The rate of the reaction for chosen concentration was measured in five replicates. Standard solutions of 0.31, 1.32, and 3.10 μg mL^{-1} of dicamba were analyzed using the recommended procedure. Five replicate determinations of each concentration gave the relative standard deviations (RSDs) of 6.10, 2.33 and 2.30%, respectively. The results obtained on five dicamba determination replicates, standard deviations, percent errors and quantitative recoveries are listed in Table 1.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were evaluated using the following equations [40, 41]:

$$LOD = 3.3 \times S_0 / b \quad (5)$$

$$LOQ = 10 \times S_0 / b \quad (6)$$

...where S_0 is the standard deviation of the calibration curve and *b* is the slope. Both limits were expressed in μg mL^{-1}. The detection limit and quantification limit of dicamba was 0.101 μg mL^{-1}and 0.306 μg mL^{-1}, respectively.

Selectivity of the Method

In order to investigate the selectivity of the proposed method, the effect of the various species on the determination of 17.0 μg mL^{-1} dicamba was studied under the optimum conditions. The maximum tolerable concentration of foreign species that produces a change in the induction period was more than ±5.0%. The results are given in Table 2. It can be seen that the majority of cations and anions did not interfere even when they are present in 1000-fold greater than dicamba. It may be seen that Cu^{2+} ions interferes a lot, showing the catalytic effect to the reaction rate. Therefore, this method has a good selectivity.

Statistical Analysis

Statistical *t*- and *F*- tests have been used to evaluate whether or not there is a significant difference between the performance of the developed and the HPLC method. Both tests were performed using MS Excel. A probability level of $p<0.05$ was considered as a statistically significant. The results obtained on three dicamba determination replicates, standard

Table 2. Effect of the foreign species on the determination of 17.0 μg mL^{-1} dicamba.

Foreign species	Tolerance level ($c_{Interferent}/c_{Dicamba}$)	
Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, SO_4^{2-}, $C_2O_4^{2-}$, NH_4^+, NO_3^-, NO_2^-	10^3	5-10
Ba^{2+}, F^-, Cl^-, SO_3^{2-}, CO_3^{2-}, As^{3+}, $S_2O_3^{2-}$	10^2	5-10
I^-, Fe^{2+}, Ni^{2+}, Zn^{2+}	10	<5
Al^{3+}, Fe^{3+}, Pb^{2+}, Cd^{2+}	1	<5
Ni^{2+}, Mn^{2+}	0.1	5-10
Cu^{2+}	0.01	<5

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Table 3. Determination of dicamba in baby food samples by kinetic and HPLC methods.

Number sample	Added dicamba (μg mL^{-1})	Determine dicamba by kinetic method[a] $\bar{x}$ ±SD (μg mL^{-1})	RSD (%)	(%)	Recovery[a] (%)	Determine dicamba by HPLC method $\bar{x}$ ±SD (μg mL^{-1})	Recovery[a] (%)	F value[b]	t value[b]
S-1	0.70	0.658±0.04	6.10	5.71	93.14	0.66±0.01	94.28	1.28	0.65
S-2	7.20	7.12±0.1	1.42	1.09	98.90	7.3±0.02	101.3	2.25	0.76
S-3	0.67	0.636±0.03	4.71	5.10	94.92	0.64±0.01	95.52	1.07	0.48
S-4	6.50	6.24±0.2	3.21	4.00	96.00	6.30±0.01	96.92	1.22	0.34
S-5	6.60	6.32±0.3	4.74	4.42	95.75	6.30±0.02	95.45	1.25	0.46
S-6	10.00	9.59±0.4	4.11	4.10	95.90	9.63±0.02	96.30	2.32	1.06
S-7	8.00	7.74±0.2	2.91	3.25	96.75	7.70±0.01	96.25	1.32	0.98
S-8	7.00	7.41±0.02	4.87	5.85	105.85	7.50±0.02	107.11	1.55	1.12
S-9	0.96	0.936±0.02	2.26	2.50	97.50	0.94±0.02	97.91	1.28	0.34
S-10	10.00	9.385±0.5	5.32	6.15	93.85	9.51±0.01	95.10	1.57	0.22
S-11	3.50	3.23±0.2	6.20	7.71	92.28	3.35±0.02	95.71	3.77	1.42
S-12	5.20	4.92±0.2	4.10	5.38	94.61	4.95±0.01	95.20	1.25	0.76
S-13	5.00	4.78±0.2	4.18	4.24	95.60	4.98±0.02	99.60	2.63	1.56
S-14	6.10	5.59±0.42	7.15	8.36	91.63	5.70±0.01	93.44	3.12	1.68
S-15	5.60	6.12±0.46	7.51	9.28	109.28	5.93±0.02	105.89	2.93	1.84
S-16	0.80	0.73±0.06	8.22	8.75	91.25	0.74±0.01	92.50	1.06	0.73
S-17	0.65	0.61±0.04	6.55	6.15	93.84	0.62±0.03	95.38	6.10	1.24
S-18	0.72	0.69±0.03	4.34	4.16	95.83	0.70±0.02	97.22	4.50	1.70
S-19	12.15	11.74±0.3	2.55	3.37	96.62	11.80±0.01	97.11	3.60	1.20
S-20	0.86	0.82±0.03	3.70	4.65	95.34	0.84±0.02	97.67	2.32	1.85
S-21	8.14	7.96±0.1	1.25	2.21	97.78	8.02±0.01	98.52	1.56	0.94
S-22	7.24	7.09±0.03	1.36	2.07	97.92	7.15±0.01	98.75	2.36	1.45
S-23	4.65	4.82±0.2	2.82	3.65	103.65	4.78±0.02	102.79	4.39	2.16
S-24	2.20	2.05±0.07	3.41	6.81	93.18	2.15±0.01	97.72	3.02	1.63
S-25	4.00	4.106±0.2	4.90	2.65	102.65	3.98±0.02	99.50	2.84	1.27

[a] Data are based on the average obtained from five determinations
[b] Theoretical F-value ($\nu_1 = 4$, $\nu_2 = 4$) and t-value ($\nu = 8$) at 95% confidence level are 6.39 and 2.306, respectively.

deviations, percent errors and quantitative recoveries obtained from linear regression equations or linear least square calibration fits are listed in Table 3.

Analysis of the Real Samples

To evaluate the analytical applicability of the proposed method, the standard addition technique according to procedure described in Experimental section was applied for the determination of dicamba concentration in spiked baby food samples. The results are shown in Table 3.

Conclusions

New kinetic method is based on the inhibited effect of dicamba in the oxidation reaction of SA by hydrogen peroxide in universal buffer (pH 9.66), in the presence of Co^{2+} ions, which behaves as a catalyst. The reaction was monitored spectrophotometrically by measuring the absorbance of formed product at 368 nm. Under optimal conditions: pH=9.66 (universal buffer), $c(Co^{2+}) = 7 \cdot 10^{-5}$ mol L^{-1}, $c(H_2O_2) = 0.25$ mol L^{-1}, $c(SA) = 4 \cdot 10^{-3}$ mol L^{-1}, t = 25 ± 0.1°C, λ = 368 nm, the method showed satisfactory standard deviation and relative standard deviation from 2.30 to 6.10 % for

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the concentration interval of dicamba from 0.31 to 3.10 μg mL^{-1}, respectively. Least-squares regression analysis used to evaluate the concentration range data indicates linearity over the interval studied (0.31-3.10 and from 3.10-31.0 μg mL^{-1}). The correlation coefficient obtained for this dicamba concentration range was –0.9977 and –0.9975, respectively. The LOD value of 0.101 μg mL^{-1} indicates that the method is sensitive. Commonly used excipients and many investigated ions were found to have no interference. The calculated recoveries of dicamba show that the proposed method is applicable and valid for analysis of baby food samples. The results obtained by kinetic method are in accordance with parallel HPLC method. Table 3 shows that the F and t values at 95% confidence level are less than the theoretical values, confirming no significant difference between the performance of the developed and HPLC method.

A new reaction system was suggested for the kinetic spectrophotometric determination of dicamba in baby food samples. This method offers several distinct advantages, namely, high selectivity and sensitivity, require cheap reagents, simple and inexpensive instruments, ease for operation, and rapidity. Statistical comparison of the results with HPLC method showed good agreement and indicates no significant difference in accuracy and precision. The kinetic method was successfully applied to determine dicamba concentrations in spiked baby food samples after solid phase extraction of the samples. The F and t values at 95% confidence level are lower than the theoretical values, confirming agreement of the developed and the HPLC method. Reliable recovery data were found at various concentrations, after spiking baby food samples, and good limits of quantification were attained. The validity and simplicity of this method allow the analysis of the baby food samples with satisfactory results.

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Conflict of Interest

The authors declare no conflict of interest.

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